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A phase II clinical trial of mefloquine in Brazilian male subjects

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Mefloquine was compared with sulfadoxine-pyrimethamine for safety and efficacy in a randomized, double-blind clinical trial in adult males from a malaria-endemic area of Brazil. A total of 99 oligosymptomatic and symptomatic volunteers with Plasmodium falciparum *parasitaemia took part in the trial; 49 were given 1000 mg of mefloquine and the remainder received 1500 mg of sulfadoxine plus 75 mg of pyrimethamine, in a single oral dose.*

Mefloquine was 100% successful in clearing parasitaemia within 7 days; there were no recrudescences. Sulfadoxine-pyrimethamine was less successful; 35 cases showed an S-type response, 8 an RI response, 3 an RII, and 2 an RIII response. The side-effects of mefloquine were mild and transient and included headache, nausea, vomiting, dizziness, and diarrhoea. A satisfactory weight gain and rise in haemoglobin level were seen in both groups.

Resistance of *Plasmodium falciparum* strains to quinine, 4-aminoquinolines, and sulfadoxine-pyrimethamine has been reported from various parts of the world, including Brazil (*1-12*, J. M. de Souza, unpublished observations, 1981). There is thus a great need for an effective, safe alternative drug for use against multiresistant malaria. Mefloquine, a quinoline-methanol derivative chemically related to quinine, was developed at the Walter Reed Army Institute of Research for the treatment of such infections (*11*), and extensive data on its pharmacology, toxicology and antimalarial activity have already been published (*12*, *13*).[a] Its usefulness in treating multidrug-resistant malaria has been reported by many investigators (*14-19*).

We have previously carried out a phase I study in adult male Brazilian volunteers from an area endemic for *P. falciparum* infection, to compare mefloquine with sulfadoxine-pyrimethamine for tolerance and safety (*20*).

The present report describes a phase II/III double-blind randomized trial on 99 adult male Brazilians from an endemic area, who had *P. falciparum* parasitaemia. Mefloquine was compared with a combination of sulfadoxine and pyrimethamine, for its effectiveness in clearing parasitaemia, safety, and side-effects. The study was carried out at the Barros Barreto Hospital, Belém, Pará, Brazil.

MATERIALS AND METHODS

A randomized, double-blind trial was carried out using a "double-dummy" system of drug administration. The duration of the study was 66 days. All patients were males over 18 years of age from the malaria-endemic area of Paragominas, and all had blood smears positive for asexual forms of *P. falciparum*. None of the subjects had received any antimalarial drugs for 4 weeks prior to admission to the study. At the time of screening and selection, none of the subjects had a fever, but some of them developed malarial fever before drug administration. Thus the trial included both oligosymptomatic and symptomatic patients with *P. falciparum* infection.

Informed consent for participation in the trial was obtained. The volunteers remained in the hospital throughout the 66-day study period.

One group of patients was given mefloquine, in the form of four tablets of 250 mg (base) each, plus 3 placebo tablets, as a single oral dose on day 0. A second group received 3 tablets of sulfadoxine-pyrimethamine (each tablet containing 500 mg of sulfadoxine and 25 mg of pyrimethamine) and 4 placebo tablets. The subjects received other drugs, such as analgesics and vitamin supplements, as required during the study.

Any relapse due to *P. vivax* was treated with chloroquine (1500 mg) and primaquine (210 mg) and recrudescence and treatment failure were treated with quinine sulfate (2 g per day for 3 days) and tetracycline (1 g per day for 3 days).

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[a] *Mefloquine hydrochloride (WR 142490 HCl); clinical brochure.* Walter Reed Army Institute of Research, Washington, DC, 1978 (unpublished document).

Before treatment, each patient gave a detailed history and received a full clinical examination according to a standard protocol, including chest X-ray and electrocardiogram (ECG). Laboratory investigations including examination of blood, haemoglobin (Hb), red blood cell (RBC) count, erythrocyte volume fraction (haematocrit), total and differential white blood cell (WBC) counts, platelet count, and reticulocyte count, were carried out on days −2 to 7, 10, 14, 21, 28, 35, 42, 49, 56, and 63. Biochemical investigations, including estimations of serum glucose, blood urea, serum bilirubin, aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), alkaline phosphatase, serum creatinine, serum cholesterol, triglycerides, calcium, sodium, potassium, chloride, magnesium, phosphate, iron, albumin, and proteins were done prior to drug administration and again on days 0, 1, 4, 7, 14, 21, 28, 42, and 63. Urine analysis was done daily during the first 10 days from day −2, and again on days 14, 21, 28, 42, and 63. Stools were examined for blood and parasites on days −2, 0, and 7. ECG studies were repeated on days −2, 0, 1, 4, 7, 28, and 63.

Blood smears were prepared and examined for malarial parasites (asexual forms and gametocytes) daily from day −2 to day 7 and then at least once a week until day 63. If patients had fever, smears were examined more frequently.

Plasma samples were collected and tested for drug level on day 7 and additionally if there was a recrudescence, or vomiting after drug administration, or in the case of a serious adverse reaction. *In vitro* drug susceptibility tests of *P. falciparum* were carried out on day 0 and again as necessary. Urine was examined for the presence of sulfonamides and 4-aminoquinolines by a modified Bratton-Marshall method and the Dill-Glazko test, respectively, prior to drug administration.

Clinical features such as pulse rate, body temperature, respiration and clinical symptoms were recorded daily. Blood pressure was recorded daily for the first 7 days and then once a week.

RESULTS

A total of 99 volunteers entered the trial, of whom 49 received mefloquine and 50 sulfadoxine-pyrimethamine. One patient from the latter group left the trial on day 2 and another on day 5, leaving 48 subjects. One further patient from this study left hospital on day 35; the remainder completed the study. In the mefloquine group, all 49 patients completed the study.

Some of the biochemical investigations, e.g., serum iron, chloride, uric acid, and magnesium were carried out in only 12–50% of cases.

In vitro studies of the sensitivity of *P. falciparum* to mefloquine and chloroquine were carried out, using the WHO standard macrotechnique, on isolates from 47 subjects.

The mean body weight of the mefloquine group was 56.1 kg (range, 41.3–76.5 kg) on day 0 and 60.4 kg (range, 45.5–81.0 kg) on day 63. There was a mean weight gain of 4.3 kg during the study. The mean body weight of the sulfadoxine-pyrimethamine group was 57.2 kg (range, 43.4–63.7 kg) on day 0 and 62.0 kg (range, 46.0–81.0 kg) on day 63. The mean weight gain was 4.8 kg. Individual variations in weight gain were wide; there was no statistically significant difference in weight gain between the two groups.

Clinical findings

Blood pressure and pulse rate showed no significant changes in either group; all ECGs remained normal. There were no significant changes in either the respiratory or neuropsychiatric systems.

Splenomegaly was seen on day 0 in 30 patients who received mefloquine and 22 patients who received sulfadoxine-pyrimethamine. There was a significant reduction in the number of enlarged spleens and in individual sizes during the 63-day study period. At the end of the study, one subject in each group still had splenomegaly. There was no significant difference between the groups. Hepatomegaly was present in 39 subjects in the mefloquine group on day 0 (2.4 cm below costal margin), and 9 subjects still had an enlarged liver on day 63 (0.3 cm). In the sulfadoxine-pyrimethamine group, 39 subjects had hepatomegaly on day 0 (2.46 cm), and on day 63, hepatomegaly persisted in 8 subjects (0.43 cm). There was no significant difference between the groups.

Laboratory investigations

In both groups, the initial mean Hb, RBC count, and erythrocyte volume fraction values were below normal, possibly as a result of previous attacks of malaria, and the helminth infections that were present in each case. In all cases, these values tended to become normal towards the end of the study.

In the mefloquine group, the mean haemoglobin value on day 0 was 6.8 mmol/litre (range, 3.92–9.7) and on day 63, 8.9 mmol/litre (range, 6.9–11.2). The mean RBC count on day 0 was 3.7×10^{12}/litre (range, 2.1×10^{12}–5×10^{12}) and on day 63 it was 4.8×10^{12} per litre (range, 4.2×10^{12}–5.4×10^{12}). The mean erythrocyte volume fraction on day 0 was 0.32 (range, 0.18–0.44) and on day 63, it was 0.4 (range, 0.34–0.5).

In the sulfadoxine-pyrimethamine group, the mean value for Hb on day 0 was 6.9 mmol/litre (range, 3.98–9.0) and 8.6 mmol/litre (range, 4.6–10.0) on day 63. The mean RBC count was

3.77×10^{12}/litre (range, 2.3×10^{12}–4.0×10^{12}) on day 0 and 4.62×10^{12}/litre (range, 3.2×10^{12}–5.0×10^{12}) on day 63. The mean erythrocyte volume fraction was 0.33 (range, 0.21–0.47) on day 0 and 0.41 (range, 0.25–0.48) on day 63.

White blood cell counts were within the normal range. Both groups had high eosinophil counts ranging from 10% to 34% of the total WBC count. No significant change was seen in total or differential WBC counts after mefloquine or sulfadoxine-pyrimethamine treatment.

None of the biochemical tests showed any drug-related changes in either group. Some cases had SGOT and SGPT values that were 10–15% higher than normal before treatment; these became normal within 1–2 weeks after drug administration. One case in the sulfadoxine-pyrimethamine group had high SGOT and SGPT levels on day 0 and day 1; these gradually subsided over the next 3 weeks. This was probably a case of viral hepatitis.

Serum iron in both groups was below normal on day 0, but reached normal values by the end of the study period.

All the subjects in both groups showed single or multiple helminth infections with *Ascaris*, hookworm, *Strongyloides*, or *Trichuris*. Associated protozoa seen in some cases were *Entamoeba histolytica*, *Giardia lamblia*, and *Endolimax nana*.

In both groups, urine samples showed traces of protein and urobilinogen in the early stages of the study. This may have been due to the destruction of red blood cells that occurs in malaria. Some cases showed an increased leukocyte count, RBC count, and casts, all of which cleared without treatment. None of the changes were related to administration of mefloquine or sulfadoxine-pyrimethamine.

The plasma levels of mefloquine, sulfadoxine and pyrimethamine will be reported elsewhere.

Parasitological response

All 49 subjects who received mefloquine had blood smears positive for asexual forms of *P. falciparum* on day 0, and all were clear by day 6 (Table 1). One patient who was clear on day 2 had a positive smear on day 7, but was again clear on day 8 without treatment. Altogether, 48 cases remained free of *P. falciparum* till day 63; one patient, who had vomited after taking the drug, had a positive smear for *P. falciparum* asexual forms on day 49. His blood level of mefloquine 6 hours after drug administration was 711 μg/litre, and on day 12 was 245 μg/litre. Thus this was probably not an RI response, but a recrudescence due to low blood drug levels.

Gametocytes of *P. falciparum* were present in 31 cases on day 0, 38 cases on day 7 and persisted in 8

Table 1. Rate of clearance of fever and parasitaemia in patients with falciparum malaria given mefloquine or sulfadoxine-pyrimethamine

Day	Mefloquine group				Sulfadoxine-pyrimethamine group			
	Fever		Parasitaemia		Fever		Parasitaemia	
	No.	%	No.	%	No.	%	No.	%
0	22[a]	100	49[b]	100	17[c]	100	48[d]	100
1	8	37	43	88	15	88	42	88
2	8	37	17	35	8	47	15	31
3	3	14	5	10	4	24	10	21
4	1	5	1	2	2	12	7	15
5	0	0	1	2	1	6	5	10.4
6	0	0	0	0	3	18	5	10.4
7	0	0	1	2	5	29.4	6	12.5
14	0	0	0	0	1	6	3	6.3
28	0	0	0	0	0	0	2	4
63	0	0	0	0	0	0	0	0

[a] Mean temperature, 37.36 ± 0.94 °C.
[b] Mean count, 10 237 ± 19 682 per mm^3.
[c] Mean temperature, 37.25 ± 0.76 °C.
[d] Mean count, 7 883 ± 24 436 per mm^3.

cases till day 28. There were no gametocytes on day 63.

Relapse due to *P. vivax* was seen in 11 cases, as shown in Table 2.

The 48 cases in the sulfadoxine-pyrimethamine group who completed the study were positive for asexual forms of *P. falciparum* on day 0, and all were clear by day 63 (Table 1). In this group, 35 cases (73%) showed an S-type response, 8 patients showed an RI response, 3 cases an RII response, and 2 cases an RIII response.

Gametocytes of *P. falciparum* were present in 35 cases on day 0, 48 cases on day 7, 20 cases on day 28, and 2 cases on day 63.

Relapse due to *P. vivax* occurred in 15 cases, as shown in Table 2.

Thus, with mefloquine the cure rate was 100%, with one recrudescence that was probably due to low blood drug levels. With sulfadoxine-pyrimethamine, the cure rate was 73%, with 8 RI, 3 RII, and 2 RIII responses. The rate of clearance of parasitaemia was quicker with mefloquine (98% on day 4) than with sulfadoxine-pyrimethamine (87.5% on day 7).

Body temperature (Table 1)

In the mefloquine group, 22 patients had fever on day 0 and all were cleared by day 5. In the sulfadoxine-pyrimethamine group, 17 cases had fever on day 0, and 16 (94%) were clear by day 5. Fever recurred in some patients on days 6 and 7 and 100% clearance was seen between days 14 and 28.

In vitro *test*

Altogether, 47 isolates were examined for sensitivity to mefloquine and chloroquine, using the *in vitro* macrotest to assess the inhibition of schizont maturation after 24 hours' incubation in the presence of different concentrations of the drugs. All patients had parasites that were resistant to chloroquine, and 9 tests showed resistance to mefloquine. The concentration of mefloquine required was 0.5–1 μmol/litre in 20 cases, 1.5 μmol/litre in 18 cases, and up to 2 μmol/litre in 9 cases.

Table 2. Number of blood smears positive for *P. vivax* in patients with falciparum malaria after treatment with mefloquine or sulfadoxine-pyrimethamine

Day	Mefloquine	Sulfadoxine-pyrimethamine
7	0	0
14	0	2
21	0	3
28	0	6
35	0	6
42	5	3
49	3	4
56	3	3
63	0	3
Total	11	15

Side-effects

All the subjects in the study were from an area where malaria, helminthiasis, intestinal protozoal infections, and malnutrition are common. Most of the volunteers had low haemoglobin, low serum albumin, enlarged spleen and liver, and helminth infections. When selected for study, all had blood smears that were positive for *P. falciparum* asexual forms, and were oligosymptomatic. However, many developed malarial fever before the drugs could be administered. Malarial fever is itself associated with headache, nausea, vomiting, dizziness, and often abdominal pain, making it difficult to differentiate between disease symptoms and drug-induced side-effects. An attempt has been made to analyse separately the symptoms in subjects with and without fever on day 0.

The side-effects occurring during the first 4 days after drug administration included headache, nausea, vomiting, mild diarrhoea, and dizziness (Table 3). All these symptoms were mild or moderate and transient. In some cases, symptomatic treatment was given. With mefloquine, the incidence of loose stools, vomiting, nausea, and dizziness was higher than with sulfadoxine-pyrimethamine. However, none of the side-effects with either drug was disturbing or serious.

Table 3. Side-effects occurring during the first four days after administration of mefloquine or sulfadoxine-pyrimethamine, in subjects with and without malarial fever

Side-effect	Mefloquine		Sulfadoxine-pyrimethamine	
	With fever	Without fever	With fever	Without fever
Headache	22/22	6/27	15/17	15/31
Dizziness	22/22	9/27	16/17	9/31
Nausea	19/22	4/27	11/17	4/31
Vomiting	7/22	2/27	4/17	0/31
Diarrhoea	9/22	6/27	6/17	3/31

DISCUSSION

The objective of this study was to compare the safety, efficacy, and tolerance of mefloquine (1000 mg) with that of sulfadoxine-pyrimethamine (1500 mg and 75 mg, respectively) given as a single oral dose to oligosymptomatic adult male subjects with falciparum malaria. The drugs were compared in terms of their rate of clearance of parasitaemia and clinical symptoms, the response of *P. falciparum* to the drug, side-effects, and changes in various haematological and biochemical factors.

The cure rate for mefloquine was 100%, compared with 75% for sulfadoxine-pyrimethamine. One case in the mefloquine group showed an RI-type response, but he had vomited soon after taking the drug, and had low blood drug levels 6 hours and 12 days after administration. In the sulfadoxine-pyrimethamine group, there were 8 cases with an RI response, 3 with an RII response, and 2 with an RIII response. The *in vitro* macrotest showed that almost all cultured parasites (from 47 cases) were sensitive to mefloquine but resistant to chloroquine.

The rate of clearance of parasitaemia and fever was faster with mefloquine than with sulfadoxine-pyrimethamine. Neither drug had any effect on gametocytes, though more gametocytes were seen after sulfadoxine-pyrimethamine treatment than after mefloquine.

The incidence of side-effects in the two groups was comparable, although there was a higher incidence of dizziness, nausea, and diarrhoea with mefloquine. However, all side-effects were mild, transient, and not disturbing and no specific treatment was needed.

Neither mefloquine nor sulfadoxine-pyrimethamine produced any adverse effects on the haematopoietic system or on liver or kidney functions.

Mefloquine, given in a single oral dose of 1000 mg, was well tolerated, safe, and highly effective. Clinically, there was no significant difference in the tolerance and safety of the drugs. However, mefloquine was more effective, producing a 100% S-type response, compared with a cure rate of 73% for sulfadoxine-pyrimethamine. In particular, this study showed that *P. falciparum* strains from this endemic region of Brazil have developed resistance to sulfadoxine-pyrimethamine. In addition, the *in vitro* macrotest showed that isolates from 47 cases had parasites that were resistant to chloroquine. Relapses due to *P. vivax* occurred after mefloquine treatment, but they appeared later than those occurring after sulfadoxine-pyrimethamine. Mefloquine has the advantage of being administered in a single oral dose, and has been shown to be safe and effective in the treatment of chloroquine-resistant falciparum malaria.

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RÉSUMÉ

ESSAI CLINIQUE DE PHASE II DE LA MÉFLOQUINE CHEZ DES BRÉSILIENS DE SEXE MASCULIN

Un essai destiné à comparer la méfloquine et l'association sulfadoxine-pyriméthamine a été réalisé de façon randomisée en double aveugle sur 97 volontaires adultes de sexe masculin originaires d'une région du Brésil où le paludisme est endémique. Tous ces sujets présentaient un paludisme à falciparum oligosymptomatique ou symptomatique et ont été admis dans le service spécialisé du paludisme à l'Hôpital Barros Barreto, Belém, Brésil. Quarante-neuf sujets ont reçu de la méfloquine à raison d'une dose orale unique de 1000 mg, et les autres ont reçu de la sulfadoxine-pyriméthamine (1500 mg de sulfadoxine et 75 mg de pyriméthamine). Tous les sujets sont restés hospitalisés pendant la période d'étude, soit 66 jours.

Dans le groupe traité à la méfloquine, 48 sujets ont présenté une réponse de type S; le sujet restant, qui avait vomi 25 minutes après avoir absorbé le médicament, avait un faible taux de méfloquine dans le sang et a présenté une recrudescence le jour 49. Dans le groupe traité à la sulfadoxine-pyriméthamine, le taux de guérison était de 73%, avec huit cas présentant une réponse de type RI, trois cas une réponse de type RII et deux cas une réponse de type RIII. La disparition de la fièvre était plus rapide avec la

méfloquine (100% en cinq jours) qu'avec la sulfadoxine-pyriméthamine (70,6% le jour 7 et 100% le jour 28). L'élimination des formes asexuées de *Plasmodium falciparum* des frottis sanguins a eu lieu en quatre jours dans 98% des cas et était totale au bout de sept jours avec la méfloquine, tandis qu'avec la sulfadoxine-pyriméthamine les taux d'élimination étaient de 85% le jour 4 et de 87,5% le jour 7.

Les effets secondaires étaient légers et passagers et consistaient en céphalées, nausées, vomissements, vertiges et diarrhées. Ils étaient légèrement plus fréquents avec la méfloquine, mais n'exigeaient aucun traitement spécifique.

Ni la méfloquine ni la sulfadoxine-pyriméthamine n'ont eu d'effet sur les gamétocytes de *P. falciparum*. Dans le groupe traité à la méfloquine, on a observé 11 rechutes dues à *P. vivax*, la première rechute étant survenue le 42^{e} jour. Dans le groupe traité à la sulfadoxine-pyriméthamine, on a observé 15 cas de rechute dus à *P. vivax*, le premier cas survenant le jour 14.

Ni la méfloquine ni la sulfadoxine-pyriméthamine n'ont eu d'effet indésirable sur les paramètres hématologiques ou biochimiques. Dans les deux groupes, on a observé une augmentation comparable du poids corporel, de l'hémoglobine et du nombre de globules rouges au cours de la période d'étude de 66 jours.

La macro-épreuve *in vitro*, réalisée sur des isolements de 47 sujets, a montré que les parasites étaient dans tous les cas résistants à la chloroquine, mais sensibles à la méfloquine.

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